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14. ABSTRACT

The term small-fiber polyneuropathy (SFPN) refers to body-wide dysfunction and degeneration of small-diameter axons that transmit pain and control the body's autonomic functions. The vague, widespread symptoms of SFPN overlap substantially with those of Gulf War Illness (GWI). We propose that there may be a SFPN component to GWI. To identify and apply the best tests to diagnose SFPN in Gulf War-ill veterans, we have been recruiting, screening, and testing normal control subjects and patients with definite SFPN to compare the sensitivity and specificity of the best current and potential new tests. We are now applying and comparing the best tests in Gulf War veterans with and without GWI to identify how often this diagnosable and treatable neurological illness is masquerading as GWI. By doing so, we will not only establish the relationship between GWI and SFPN, but will also determine which tests are the most diagnostically useful. This report summarizes progress against Specific Aims I and II of the basic statement of work.

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INTRODUCTION:

The term small-fiber polyneuropathy (SFPN) refers to body-wide dysfunction and degeneration of the small-diameter axons that transmit pain and control the body's autonomic (involuntary) functions. The vague, widespread symptoms of SFPN overlap substantially with those of Gulf War Illness (GWI). SFPN is hard to diagnose clinically and requires special tests. We propose that there may be a SFPN component to Gulf War Illness. To identify and apply the best tests to diagnose SFPN in Gulf War-ill veterans, we have been recruiting, screening, and testing normal control subjects and patients with definite SFPN to compare the sensitivity and specificity of the best current tests (skin biopsy and comprehensive autonomic-function testing (AFT)), as well as a potential new test (axon flare reflexes to vasodilators) (Aim I). We are now applying the best of these tests to compare results in Gulf War veterans with and without Gulf War illness to identify how often this diagnosable and treatable neurological illness is masquerading as Gulf War Illness (Aim II). By doing so, we will not only establish the relationship between Gulf War Illness and SFPN, but will also determine which tests are the most diagnostically useful and should be adapted for more widespread clinical use. This report summarizes progress against Tasks 1, 2, and 3 of Specific Aims I and II of the basic statement of work which is included at Appendix 1.

PROGRESS AGAINST SPECIFIC AIMS:

The initial phase of this study was aimed at establishing normative neurite densities from a diverse normal population and identifying the test(s) of greatest utility in diagnosing small-fiber polyneuropathy, which has been identified as a strong need in the neurology community [1]. We have made a preliminary determination of the best tests and are currently recruiting and testing Gulf War veterans with those tests. Progress against the sub-tasks scheduled to be accomplished or begun during the first two years this study (a, b, and c of Task 1; and a, b, c, and d of Task 2 of Specific Aim I; and a and b of Task 3 of Specific Aim II) is detailed below. Please refer to the Statement of Work, included at Appendix 1, for the complete study plan.

Specific Aim I. To determine which specific measurements of skin innervation, autonomic function, and skin blood flow provide the most sensitive, specific, and practical objective test for SFPN.

Task 1. Establish demographically correct skin biopsy norms. A cohort of 120 normal controls will be established to provide the necessary range of ages, sexes and ethnicities

a. Recruit, screen and test 120 normal controls. Some subjects have already been studied to provide preliminary data for this application. (months 1 – 6)

b. Multivariate data analysis to determine which of the three demographic variables tested (age, sex, race/ethnicity) influences the normal values for density of skin innervation and to generate the norms and limits between the normal and abnormal ranges necessary for clinical diagnostic use. (months 6 – 8)

c. To prepare and publish a manuscript in a high-impact neurological journal that will make these norms available for medical use world-side. An internet version will also be made available. (months 8 – 20)

Task 1 involves establishing demographically normal values for density of innervation in the distal-leg skin biopsies used to diagnose small-fiber polyneuropathy. Accurate diagnosis of disease depends entirely on accurate definitions of the normal range. Early studies of skin neurite quantification by skin biopsy [2] established a cutoff value below which a neurite density was considered abnormal regardless of age, gender, or ethnicity. This was considered the standard of diagnosis until more recent work which has begun to show evidence of differences in neurite density by age and gender [3]. A major shortcoming of these studies, including a world-wide collaboration of neurite density databases [4], is that the study populations were either not diverse or they did not identify ethnic differences in neurite density in addition to gender differences. Our study expands on these findings by studying a cohort of at least 120 normal control subjects of various ages, genders, and ethnicities.

Methods: We recruited normal subjects primarily through in-house advertisement and through the Research Study Volunteer Program (RSVP for Health) administered by our Clinical Research Program. All normal subjects were initially telephone-screened to rule out confounding health issues. In addition, normal subjects first underwent a 2-hour fasting glucose-tolerance test (GTT) to rule out occult diabetes, which is increasingly prevalent and carries high risk of polyneuropathy.

Skin biopsies are performed in our JCAHO-accredited laboratory. After informed consent, a site (10 cm above the ankle) is anesthetized and one or two 2- or 3mm diameter skin punches are removed using sterile technique and the site covered with a Band-Aid. Samples are immediately fixed and then sectioned (using a freezing sliding microtome) and processed using standard methods. For PGP9.5-IR, Zamboni's fixative, 50 micron sections, and a 1:1200 dilution of polyclonal antibody specific for the pan-axonal enzyme ubiquitin hydrolase (PGP9.5) are used, followed by standard DAB labeling. For each biopsy, a single morphometrist then counts the total number of separate immuno-positive neurites within the entire epidermis which is then normalized to the skin surface area to yield a neurite density per square millimeter of skin surface.

We engaged statistical assistance through the Biostatistics Unit of Mass General Hospital's Clinical Research Program to review our findings and to develop the final multivariate regression analysis to establish normative skin biopsy values.

Outcomes: Task 1a: We had already studied the 120 normal controls during Year 1 of this study but continued to add to that total because (1) we were able to pool results of prior studies in our laboratory (using identical techniques) with those obtained under this study and (2) we also had to recruit additional controls because we needed additional subjects who could undergo the complete set of tests for Task 2 (glucose tolerance test, AFT, skin biopsy, axon flare), and many of the subjects who were partially studied previously were no longer available to complete all required study tests. By the end of calendar year 2011 we had studied 215 subjects aged 18 and older. Our actual number of subjects studied was greater, but we removed 14 subjects from the

analysis either for pre-existing conditions that were not disclosed during the initial screening, or once it was demonstrated by 2-hour fasting glucose tolerance test that they had impaired glucose tolerance (i.e., pre-diabetes), as defined by criteria of the American Diabetes Association. The age distribution of the included subjects is summarized in Table 1.

Age Range	No.
18-19	18
20-29	56
30-39	38
40-49	33
50-59	26
60-69	26
70-79	12
80-89	6
Total	215

Table 1. Distribution of normal subject ages

Task 1b: In addition to the adult normal controls listed in Table 1, we also biopsied 25 youngsters aged 14-17 through other studies, for a total of 240 subjects. Although pediatric norms are outside of the scope of this study, the biopsy results of the youngsters anchor the lower end of the normal biopsy curve from which the multivariate analysis was derived. Thus, their biopsies remain part of the data set for the analysis of this study even though they were not studied under this grant. For the purpose of advancing the analysis of Task 1b, we discontinued adding to the normal dataset as of the end of calendar year 2011, even though more biopsies were being collected through the rest of Year 2 of this study. The full data set is presented in Figure 1 where the youngsters (under age 18) are in red.

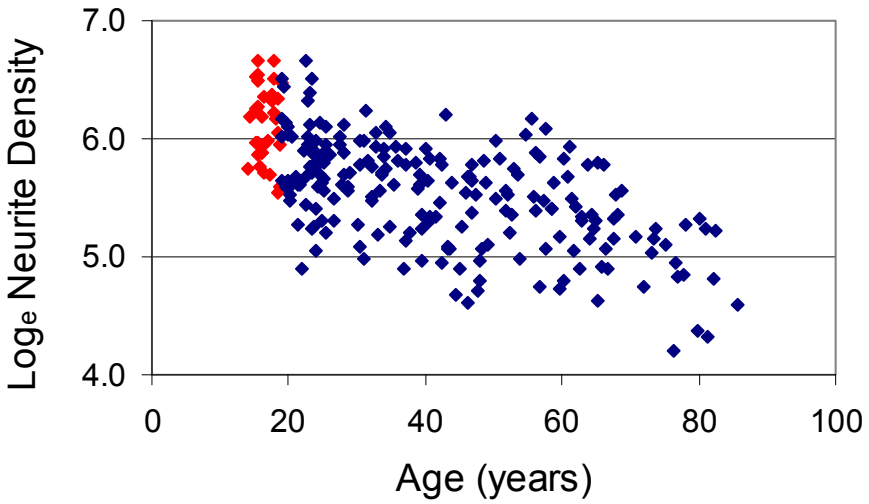

Figure 1. Logarithmic plot of neurite densities of 240 normal controls. Subjects under age 18 (not part of this study, but included in the analysis) are in red

We confirmed the preliminary analysis of Year 1 results, that among our normal controls females in general had significantly higher neurite densities than males. We also observed that Asian subjects had significantly higher neurite densities than non-Asian subjects. We found that there was also a difference between Black and non-Black subjects, but our population was insufficiently powered in Hispanic/Latino, or Hawaiian/Pacific Islanders to show any significant differences among those populations. The ethnic composition of the final normal cohort is presented in Table 2, and graphically in Figure 2.

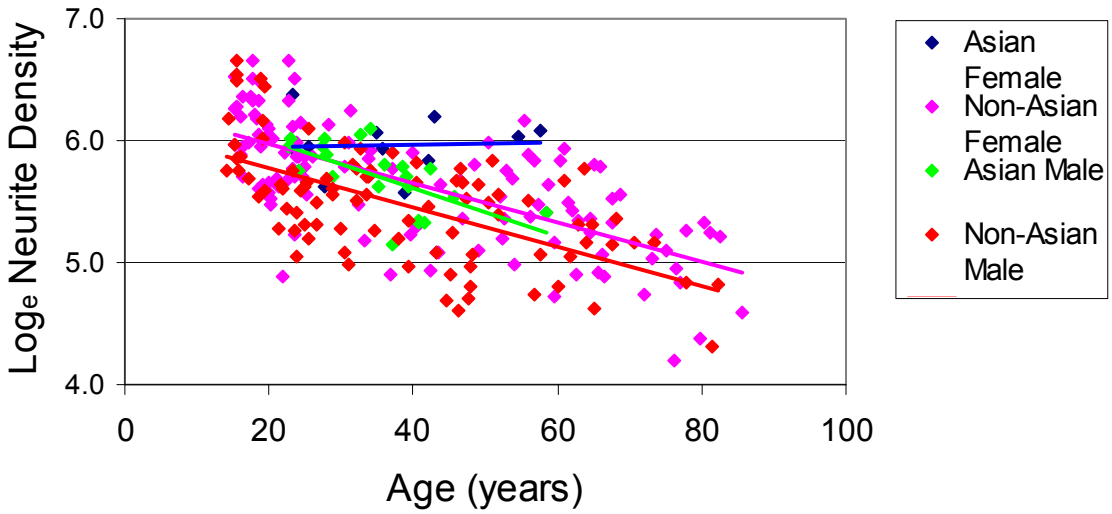

Figure 2. Ethnic composition of the normal control cohort for skin biopsy

We engaged Dianne Finkelstein, who directs MGH's Biostatistics Unit to develop the final multivariate regression analysis of our results. We previously observed that a logarithmic scale provided the best linear regression fit and that young subjects had a superabundance of neurites that tended to skew that linear regression fit. We concluded that the best log data fit was to a "bimodal" model with a break point at age 22, i.e., data are analyzed separately for best fit below age 22 and above age 22. The statisticians thus fitted a "piecewise" linear regression model for log density as a function of age, gender, and ethnicity, with a change point at age 22. The model fit was confirmed with an analysis of the residuals (for normality and homoscedasticity). The model fit (showing gender differences only) is shown in Figure 3.

Ethnic populations	Male	Female
White	80	105
Asian	17	11
Indian subcontinent	4	0
Black	5	10
Hispanic	5	1
Pacific Islander	1	1
Non-Asian	91	117
All Asians	21	11
Total	240	

Table 2. Ethnic populations in the skin biopsy cohort. These totals include young subjects aged 14-17.

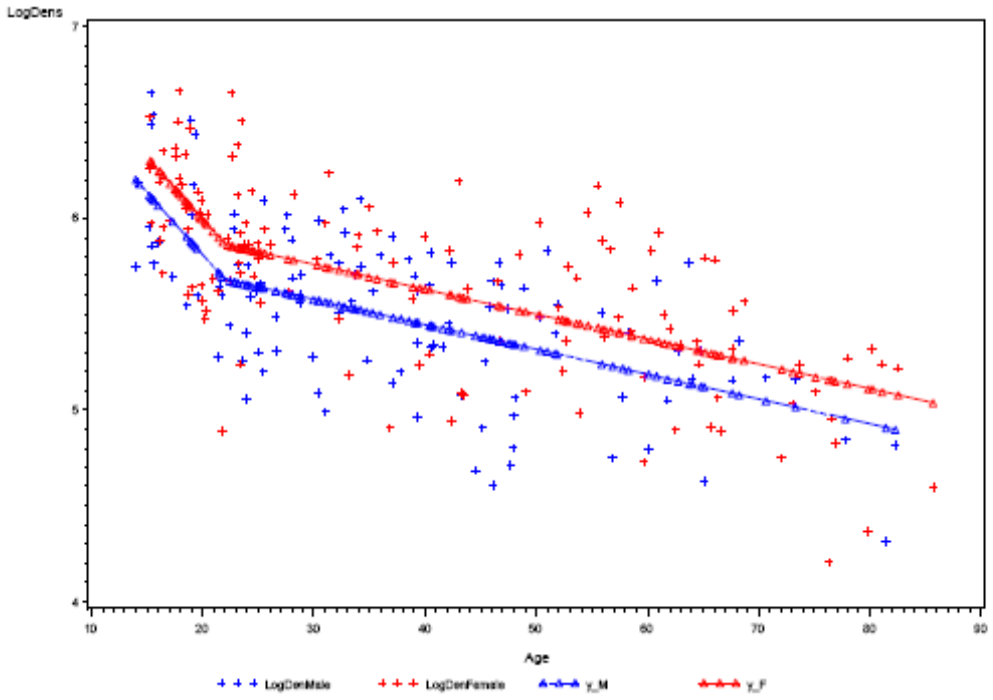

Figure 3. Log_n density vs. age with breakpoint at age 22.
Upper line (red) is female, lower line (blue) is male

Thus a multivariate regression analysis that accounts for the variations attributed to age, gender, and ethnicity with a break point at age 22 is appropriate and is presented in Equation (1). We will use this regression analysis going forward even though we will still be obtaining biopsies from the remaining osteoarthritis subjects (see Task 2, below), who are considered normal (non-neuropathy).

Equation (1):

$$Log_n \text{ (neurite density)} = 7.06 - 0.064(\text{age}) + 0.051(\text{age} - 22) + 0.201(\text{gender}) + 0.253(A) - 0.235(B)$$

Where
neurite density is in neurites/mm^2
age is in years,
gender = 1 if female, 0 if male,
A = 1 if Asian, otherwise = 0,
B = 1 if Black, otherwise = 0

Task 1c. As we have completed Tasks 1a and 1b of the study, a manuscript is being prepared for publication of these results. Availability of the biostatisticians was a limiting factor for the analysis that is central to the manuscript. We expect to submit the manuscript for publication early in the first quarter of Year 3.

To make these results accessible to the scientific and clinical community we have also developed a neurite density calculator based on the multivariate regression analysis. We plan to install it on

our laboratory web site for use by researchers and diagnosticians who wish to verify a diagnosis of small-fiber neuropathy or obtain a predicted value for patients with specific demographics. A sample neurite density calculator screen is shown in Figure 4. Entering Age, Gender, and Race returns an expected normal neurite density ("Predicted Neurite Density value"). However, if a neurite density (in neurites per mm^2 of skin surface) is already known and is entered ("Enter raw Neurite Density") then a Percentile is returned which compares that neurite density with the normal population of our study. The diagnostician may then interpret normal and abnormal range values based on the percentile. It is common practice to interpret neurite densities at or below the 5th percentile as abnormal and indicative of neuropathy.

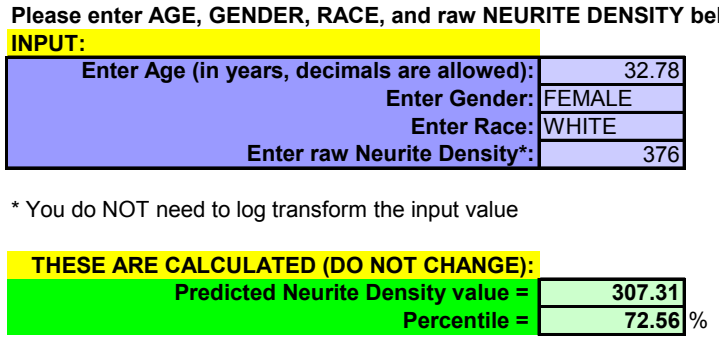

Figure 4. Sample screen shot of the Neurite Density Calculator

Task 2. Compare the diagnostic sensitivity and specificity of skin biopsy, AFT, and axon-flare measurements to establish best tests for SFPN. Data will be collected from cohorts of 40 screened normal volunteers, SFPN patients, and symptom-matched control patients with severe osteoarthritis.

a. Recruit 40 normal subjects from among the 120 being studied by skin biopsy for Aim I for additional study with AFT and axon-flare measurements. (months 3 – 12)

b. Recruit 40 subjects with definite SFPN from among the several hundred already evaluated for clinical care at Mass. General Hospital by skin biopsy and AFT for additional study of axon-flare measurements. (months 8 – 18)

c. Recruit 40 severe osteoarthritis of the hip or knee from among the thousands such patients followed at Mass. General Hospital for study by skin biopsy, AFT, and axon-flare measurements. (months 8 – 18)

d. Multivariate data analysis to determine which of the tests have greatest potential for clinical diagnostic use. Positive and negative predictive value, diagnostic sensitivity and specificity, invasiveness and cost will be considered. Tests that complement or overlap will be identified. (months 18 – 22)

Task 2 provides the data to establish the best test for SFPN, which has been identified as a diagnostic need [1]. We are comparing the results of 3 diagnostic tests for SFPN (skin biopsy,

AFT, and axon flare) to identify which has the best predictive value. In addition, we are ruling out other potential sources of SFPN through questionnaire and 2-hour fasting glucose tolerance test (GTT). One study that indicated a possible correlation between axon flare size, sweat production (one of the AFT tests), and neurite density did not include a control comparison group [5]. We are studying osteoarthritis subjects as an additional, positive control group to demonstrate that although the osteoarthritis subjects are also experiencing chronic pain, as do the SFPN patients, their pain has a non-neuropathic origin, and thus peripheral nerve test results from the osteoarthritis subjects should not differ significantly from the normal controls. To our knowledge this is the first study to use this approach.

Methods: We have been recruiting subjects mainly through in-house advertisement and through the Research Study Volunteer Program (RSVP for Health) administered by our Clinical Research Program. To the extent possible for normal controls, we invited previously studied subjects to continue in this study. Small-fiber polyneuropathy (SFPN) subjects from the neurology practice at Massachusetts General Hospital were also invited to participate if they received a definite diagnosis of SFPN from a neurologist. We are also recruiting additional osteoarthritis subjects through their physicians, aided by automated searches through Mass General Hospital's Research Patient Data Registry (RPDR) which is a searchable clinical data repository for patients treated within the Partners Healthcare (the parent organization of MGH and affiliated hospitals) system.

Autonomic Function Testing (AFT) consists of four specific separate tests that are routinely used and endorsed for clinical diagnostic testing for small-fiber polyneuropathy. AFT is administered by personnel trained by the manufacturer (WR Medical Electronics, Stillwater, MN). The specific tests are (1) QSART (quantitative sudomotor axon reflex test) where sweat production is quantitated from the standard forearm, proximal leg, distal leg, and foot sites in response to iontophoresis of acetylcholine; (2) heart rate response to deep breathing where heart rate variability during inspiration and expiration is measured for at least 5 cycles; (3) beat-to-beat heart rate and blood pressure responses in phases II and IV of the Valsalva maneuver where heart rate variability is measured while the subject is asked to blow into a bugle to maintain a column of mercury between 40 and 50 mm; and (4) beat-to-beat heart rate and blood pressure responses to tilt where continuous blood pressure and heart rate monitoring is performed for 5 minutes with the subject supine, then the subject is placed in an 80 degrees heads-up tilt position for 10 minutes and subsequently returned to supine for an additional 5 minutes of recording.

Laser Doppler measurements of skin blood-flow and axon flare are performed in our laboratory using equipment from Moor Instruments Ltd, Devon, UK. Changes in blood flow rate are measured in response to a vasodilator (histamine or acetylcholine) which is introduced into the skin via iontophoresis. A hollow plastic ring is affixed to a subject's skin which positions two fiber-optic laser leads, and is also a reservoir for the vasodilator. After measuring baseline blood flow, a current is applied to the liquid reservoir in the ring to drive the charged vasodilator molecules into the skin. Axon flare intensity measurements are taken continuously over time while a laser-Doppler imager provides a sequence of blood flow (flux) maps, taken at predetermined intervals.

Outcomes: Tasks 2a, 2b, and 2c: Table 3 summarizes the progress of normal, osteoarthritis, and SFPN subject recruitment under Task 2. All 40 Normal Control and SFPN subjects have been studied with the full range of tests. 28 osteoarthritis controls have undergone the full range of tests and 3 others have nearly completed the study. The primary difficulty in recruiting osteoarthritis controls is that, as a condition mostly of the elderly and/or overweight, there is a greater prevalence of impaired glucose tolerance (IGT) or other confounding conditions. We rejected 12 osteoarthritis subjects from the study for IGT or other pre-existing conditions. All 40 SFPN subjects have been studied with the full range of tests.

	Skin Biopsy	AFT	GTT	Axon Flare
No. of Normal Control Subjects (target 40)				
40	✓	✓	✓	✓
No. of Osteoarthritis Controls (target 40)				
28	✓	✓	✓	✓
2	✓	✓	✓	
1		✓	✓	✓
No. of SFPN subjects (target 40)				
40	✓	✓	✓	✓

Table 3. Status of subjects having undergone multiple tests under Task 2

Progress against 2d. Study of the full cohort of osteoarthritis subjects has not been completed yet but a preliminary analysis indicates that autonomic function test and skin biopsy are both superior in predictive value and reproducibility than measurement of axon flare. In order not to delay this project further while awaiting test of the remaining osteoarthritis controls, we have proceeded with recruitment and test of Gulf War veterans (Specific Aim II) with both AFT and skin biopsy with the understanding that one of these tests may ultimately prove superior in diagnostic value, or we may find that both tests are necessary. This is in line with researchers who have done similar comparisons of diagnostic methods and advocate a multi-modal approach to diagnosis of SFPN [6]. Progress against Specific Aim II is described in the next section.

Specific Aim II. To use the best of these tests to determine the prevalence of SFPN among GW-ill veterans recruited with the assistance of the VA Decision Support System, and to compare SFPN prevalence to the prevalence in unaffected Gulf-War veterans and our demographically matched civilian controls.

Task 3: Determine prevalence of SFPN in Gulf War-ill veterans. The best tests identified above will be administered to groups of normal Gulf War veterans and veterans suffering from Gulf War Illness.

a. Recruit healthy and ill Gulf War veterans. Cohorts of 150 of each veteran group will be recruited by a combination of electronic medical-record searches at Mass. General Hospital, VA databases, and DoD databases. Additional IRB approvals external to MGH may be required. (months 18 – 30)

b. Test veteran cohorts with best test(s) of Task 3 to determine prevalence of SFPN among Gulf War Ill veterans. (months 22 – 30)

Methods: Task 3a. We have been recruiting Gulf War veterans using multiple strategies. We began recruiting veterans through in-house advertisement and through RSVP for Health. We posted IRB-approved flyers to further advertise this study and contacted other researchers working with Gulf War veterans to share strategies and to mutually promote our respective

studies. We performed RPDR searches to identify veterans of the appropriate age to have served in the first Gulf War followed by free-text searches for mention of Gulf War service. We also contacted the Defense Manpower Data Center (DMDC) Data Request System (DRS) to conduct a search of military personnel who served in the first Gulf War and currently reside in the New England area (specifically Massachusetts, Rhode Island, New Hampshire, Maine, Vermont, Connecticut, and New York). We began a manual cross-reference with Partners HealthCare's Longitudinal Medical Records (LMR) to identify the physicians of those Gulf War veterans who have been examined or treated at an affiliated Partners Healthcare hospital. IRB regulations only allow us to contact veterans through their physicians. We have an IRB-approved letter of introduction that their physician can then forward about our study. We also engaged information technology assistance from the RPDR administrators to devise a semi-automated search that would cross-reference the DMDC dataset with RPDR records. We contacted approximately 100 Veterans Service Organizations to promote our study. We are also working with the National Service Officer and National Area Supervisor for Disabled American Veterans of Massachusetts who is including our study brochure in mailings to Massachusetts veterans. We tried to advertise the study through area Veterans Health Administration (VHA) hospitals. We are also investigating gaining access to the Devens cohort of Gulf War veterans.

Task 3b. The study methods for skin biopsy and autonomic function test are the same as described in Task 2. In addition, we have added an additional study instrument, a brief neurological exam, the Utah Early Neuropathy Scale [7], that was specifically designed and validated for neuropathy detection. It numerically rates motor function, reflex, pin sensation, large fiber sensation, and allodynia/hyperesthesia. This will help us to better quantify the degree of neuropathy of each subject for comparison. Veterans are asked to provide their Service/Discharge record (DD 214) and, to be eligible for inclusion in the Gulf War ill group, they must produce either VA Persian Gulf Registry Form 10-9009a or the resulting letter from the VHA physician attesting to their diagnosis of Gulf War illness.

Outcomes: Task 3a. The initial RPDR search took great time and effort and did not net many subjects so far. The DMDC search returned a dataset of 17,926 Gulf War veterans currently residing in the New England area; 3,192 in Massachusetts alone. The cross-reference search of these records with RPDR identified 730 veterans who were treated at MGH or Brigham and Women's Hospital (BWH). Of those, the search found physicians for 286 veterans; 444 had no physician of record. Further manual searches of the electronic medical records will be needed to identify them. Disabled American Veterans of Massachusetts identified 1,100 veterans in Massachusetts and we are in the process of assisting with preparing the mailing that includes our study brochure. Recruiting through local VHA hospitals was not successful because they asked that we first apply for permission through their individual IRBs, even to post our study brochures. Several Veterans Service Organizations were helpful, however.

Task 3b. We have studied 24 Gulf War veterans to-date, 4 with verified Gulf War illness (GWI) and 20 “controls”. Among them, 10 were indeed healthy and free of any GW symptoms. However, as shown in Table 4, 10 (50%) have multi-symptom health complaints that they attribute to GWI, despite no official VHA diagnosis of GWI. These “ill controls” are disproportionately participating in our study. Thus, an unanticipated third subject group has emerged that necessitates a reconsideration of the study design. These symptomatic controls are

not representational of the population that served in the Gulf War, and unless we change the originally planned study design, we might obscure true differences between the Gulf War ill and normal subjects. While the "symptomatic controls" are of interest, they need to be distinguished from true "normal controls".

Category	Studied
Gulf War ill veterans	4
Healthy Gulf War veterans	10
Symptomatic Gulf War veterans without diagnosis	10
Total	24

Table 4 Gulf War veterans tested as of the end of Year 2

To do so, we will be adding additional study tools. These have been IRB-approved at MGH for other studies. We have added the Utah Early Neuropathy Scale (UENS, previously discussed), and we are investigating additional study instruments (questionnaires, examinations) that can be added to the study to better understand the health of this symptomatic group and to establish an additional case history for them. Additional study instruments under consideration are the Beck Depression Inventory, Visual Analog Scale for pain, the Short Form McGill Pain Questionnaire (SF-MPQ-2), the Michigan Neuropathy Screening Instrument (which includes elements of the UENS), and the SF-36 health survey questionnaire.

KEY RESEARCH ACCOMPLISHMENTS:

- Young normal control subjects (below age 22) were found to have a "superabundance" of skin neurites.
- Normal individuals were shown to have significant racial differences in cutaneous neurite densities.
- Female normal controls were found to have significantly higher neurite densities in skin than males.
- A logarithmic piece-wise linear regression model of the normative skin-biopsy data that factors in sex, race, age was developed.
- This model was used to develop a neurite density calculator to provide more accurate norms and centiles for individual patients.

REPORTABLE OUTCOMES:

This project has only recently begun to study Gulf War veterans, but substantial groundwork was done to establish the appropriate tests and normative data to make such a study meaningful. Work that culminated in several abstracts prior to this study were carried over into this work to add significance to the findings. For instance, we presented preliminary results that indicated a superabundance of skin neurites in youngsters and a dependence of skin neurite density on age, gender, and ethnicity [8]. This work was awarded a Works in Progress designation by the American Neurological Association which identifies significant late-breaking research. We also retrospectively and prospectively explored which diagnostic tests may have better predictive value for small fiber polyneuropathy among a small initial cohort of SFPN patients and normal

controls [9]. We have constructed an automated neurite density calculator based on the normative neurite density values that we have collected, which we will make available to researchers and clinicians. A manuscript is in preparation to report these neurite density findings [10].

CONCLUSION:

In its first two years this study has established a new normative scale of neurite density from skin biopsy based on age, gender, and ethnicity, that will globally improve skin-biopsy diagnosis of small fiber polyneuropathy. We have identified the need for both skin biopsy and autonomic function testing as objective testing for SFPN diagnosis. We have developed successful recruitment strategies to study veterans of the first Gulf War with punch skin biopsy and autonomic function test. We have identified a new cohort of veterans (symptomatic but not officially diagnosed with Gulf War illness) that will need additional consideration and modification of study design, to be resolved in Year 3.

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Appendix 1

Statement of Work under W81XWH-10-1-0534

The timeline at page 3 of the Statement of Work has been updated to show progress of the individual subtasks. Green triangles indicate completed tasks, yellow is tasks in progress, and gray includes those tasks beyond Year 2.

STATEMENT OF WORK

Undiagnosed small-fiber polyneuropathy - Is it a component of Gulf-War Illness?

Background: The term small-fiber polyneuropathy (SFPN) refers to body-wide dysfunction and degeneration of the small-diameter axons that transmit pain and control the body's autonomic (involuntary) functions. SFPN typically causes chronic pain, gastrointestinal symptoms, fatigue, dizziness, chronic headache, and skin abnormalities - complaints that overlap substantially with Gulf War Illness (GWI). Because SFPN produces vague, widespread symptoms, it is hard to diagnose clinically and requires special tests. In Aim I we will recruit, screen, and test normal control subjects and patients with definite SFPN from among the hundreds seen at Mass. General to compare the sensitivity and specificity of the best current tests (skin biopsy and comprehensive autonomic-function testing (AFT)), as well as a potential new test (axon flare reflexes to vasodilators). In Aim II, we will apply the best of these tests and compare results in Gulf War veterans with and without Gulf War illness to identify how often this diagnosable and treatable neurological illness is masquerading as GWI. By doing so, we will not only establish the relationship between Gulf War Illness and SFPN, but will also determine which tests are the most diagnostically useful and should be re-engineered for more widespread clinical use. Most procedures in this study, including access to patient records, telephone screening, and skin biopsy and axon flare testing are already approved by the Partners Human Research Committee's Institutional Review Board (IRB) under protocol #1999-P-009042, "Laboratory Evaluation of Neuropathic Pain".

Specific Aim I. To determine which specific measurements of skin innervation, autonomic function, and skin blood flow provide the most sensitive, specific, and practical objective test for SFPN.

Task 1. Establish demographically correct skin biopsy norms. A cohort of 120 normal controls will be established to provide the necessary range of ages, sexes and ethnicities

a. Recruit, screen and test 120 normal controls. Some subjects have already been studied to provide preliminary data for this application. (months 1 – 6)

b. Multivariate data analysis to determine which of the three demographic variables tested (age, sex, race/ethnicity) influences the normal values for density of skin innervation and to generate the norms and limits between the normal and abnormal ranges necessary for clinical diagnostic use. (months 6 – 8)

c. To prepare and publish a manuscript in a high-impact neurological journal that will make these norms available for medical use world-side. An internet version will also be made available. (months 8 – 20)

Task 2. Compare the diagnostic sensitivity and specificity of skin biopsy, AFT, and axon-flare measurements to establish best tests for SFPN. Data will be collected from cohorts of 40 screened normal volunteers, SFPN patients, and symptom-matched control patients with severe osteoarthritis.

a. Recruit 40 normal subjects from among the 120 being studied by skin biopsy for Aim I for additional study with AFT and axon-flare measurements. (months 3 – 12)

b. Recruit 40 subjects with definite SFPN from among the several hundred already evaluated for clinical care at Mass. General Hospital by skin biopsy and AFT for additional study of axon-flare measurements. (months 8 – 18)

c. Recruit 40 severe osteoarthritis of the hip or knee from among the thousands such patients followed at Mass. General Hospital for study by skin biopsy, AFT, and axon-flare measurements. (months 8 – 18)

d. Multivariate data analysis to determine which of the tests have greatest potential for clinical diagnostic use. Positive and negative predictive value, diagnostic sensitivity and specificity, invasiveness and cost will be considered. Tests that complement or overlap will be identified. (months 18 – 22)

e. To prepare and publish a manuscript in a high-impact neurological journal that will make these recommendations available for medical use world-side. (months 22 – 34)

Specific Aim II. To use the best of these tests to determine the prevalence of SFPN among GW-ill veterans recruited with the assistance of the VA Decision Support System, and to compare SFPN prevalence to the prevalence in unaffected Gulf-War veterans and our demographically matched civilian controls.

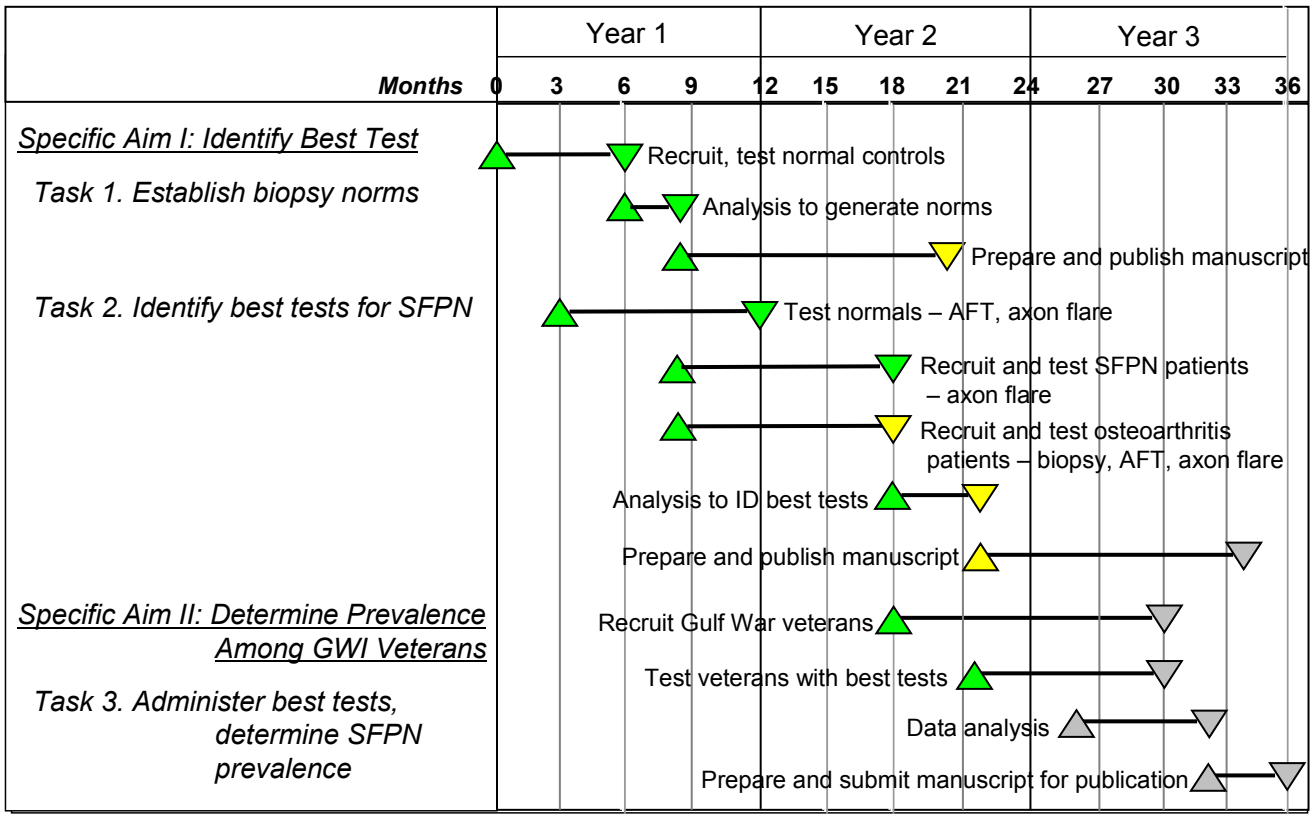
Task 3: Determine prevalence of SFPN in Gulf War-ill veterans. The best tests identified above will be administered to groups of normal Gulf War veterans and veterans suffering from Gulf War Illness.

c. Recruit healthy and ill Gulf War veterans. Cohorts of 150 of each veteran group will be recruited by a combination of electronic medical-record searches at Mass. General Hospital, VA databases, and DoD databases. Additional IRB approvals external to MGH may be required. (months 18 – 30)

d. Test veteran cohorts with best test(s) of Task 3 to determine prevalence of SFPN among Gulf War Ill veterans. (months 22 – 30)

e. Data analysis to determine and compare the prevalence of SFPN in Gulf War ill and controls. Multivariate data analysis to determine which of the tests have greatest potential for clinical diagnostic use. Positive and negative predictive value, diagnostic sensitivity and specificity, invasiveness and cost will be considered. Tests that complement or overlap will be identified. (months 26 – 32)

f. To prepare and submit for publication a manuscript in a high-impact medical journal that will make these findings available world-side. (months 32 – 36)


Year 1
Year 2
Year 3
Months
0 3 6 9 12 15 18 21 24 27 30 33 36
Specific Aim I: Identify Best Test
Task 1. Establish biopsy norms
Recruit, test normal controls
Analysis to generate norms
Prepare and publish manuscript
Task 2. Identify best tests for SFPN
Test normals – AFT, axon flare
Recruit and test SFPN patients – axon flare
Recruit and test osteoarthritis patients – biopsy, AFT, axon flare
Analysis to ID best tests
Prepare and publish manuscript
Specific Aim II: Determine Prevalence Among GWI Veterans
Recruit Gulf War veterans
Test veterans with best tests
Task 3. Administer best tests, determine SFPN prevalence
Data analysis
Prepare and submit manuscript for publication